TABLE III. Clinical Characteristic According to Genotypes

	Genotype 1a (N = 32)	Genotype 1b (N = 945)	*P*-value
Age (y.o.)	36.4 ± 2.2	55.9 ± 11.6	0.0001
Sex: male/female	28/4	546/408	0.0004
Patients with hemophilia	23	4	0.0001
AST (IU/L)	48.8 ± 33.6	59.9 ± 45.0	0.1745
ALT (IU/L)	64.6 ± 57.8	64.6 ± 57.8	0.9894
Platelet (10^4/μl)	18.8 ± 6.0	17.2 ± 6.0	0.0918
HCV levels (KIU/ml)	2607.4 ± 3072.2	2011.5 ± 1453.8	0.0642

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus.

lack of response to IFN therapy. Frequency of early virological response, characterized by undetectable HCV at 12 weeks, was 30.4% (7/23). Virological response rate at the end of treatment was 47.8% (11/23). Finally, 11 of 23 patients (47.8%) achieved sustained virological response. Clinical characteristics were compared between patients who achieved sustained virological response and patients who did not (Table IV), revealing significant differences in two factors on univariate analysis: IL28B and ISDR.

DISCUSSION

The present study investigated 977 patients with genotype 1 using direct sequencing of core and NS5A regions, revealing that genotype 1a is rare (3.3%) in Japan. Of the 33 patients with genotype 1a, 23 (71.9%) were patients with hemophilia, confirming that the majority of cases with genotype 1a involve patients with hemophilia who have received imported clotting factors, as previously reported [Fujimura et al., 1996; Otagiri et al., 2002; Hayashi et al., 2003]. Analysis after excluding patients with hemophilia revealed the prevalence of genotype 1a in Japan was 0.9% (9/954). Recently, the distributions of HBV genotypes have been changing in Japan due to international exchange [Hayashi et al., 2007; Matsuura et al., 2009]. However, prevalences of HCV genotypes have remained stable because of the different modes of infection involved. The present study revealed that 11 (47.8%) of 23 patients achieved sustained virological response. The IFN responsiveness of HCV genotype 1a in Japanese patients was reported in 1999 from Okinawa, a far southern island in Japan [Sakugawa et al., 1997]. That study reported that the rate of sustained virological response tended to be higher in patients with genotype 1a than in those with genotype 1b, but no significant differences were identified because of the small number of patients with genotype 1a. Low virological response rates in both genotypes 1a and 1b were confirmed in the present Japanese patients, as in Caucasian patients [Manns et al., 2001; McHutchison et al., 2009]. No significant differences in sustained virological response rate were seen between genotypes 1a and 1b. Discriminating between genotypes 1a and 1b thus seems to have little clinical relevance in terms of IFN responsiveness. Viral factors associated with sustained virological response, including HCV genotype, have been studied most frequently studied and mutations in the core and NS5A regions of HCV genotype 1b have been associated with response to IFN therapy [Akuta et al., 2005, 2010, 2011; Okanoue et al., 2009; Nakagawa et al., 2010; Toyoda et al., 2010; Hayashi et al., 2011a; Hayes et al., 2011; Kumthip et al., 2011; Kurosaki et al., 2011]. These viral factors could improve prediction of sustained virological response for genotype 1a, as in 1b. Amino acid substitutions at positions 70 and 91 of the HCV core region in genotype 1b have been related to IFN responsiveness, liver steatosis, hepatic oxidative stress, insulin resistance, and carcinogenesis [Akuta et al., 2005, 2007, 2009; Tachi et al., 2010]. These substitutions may have substantial impacts on

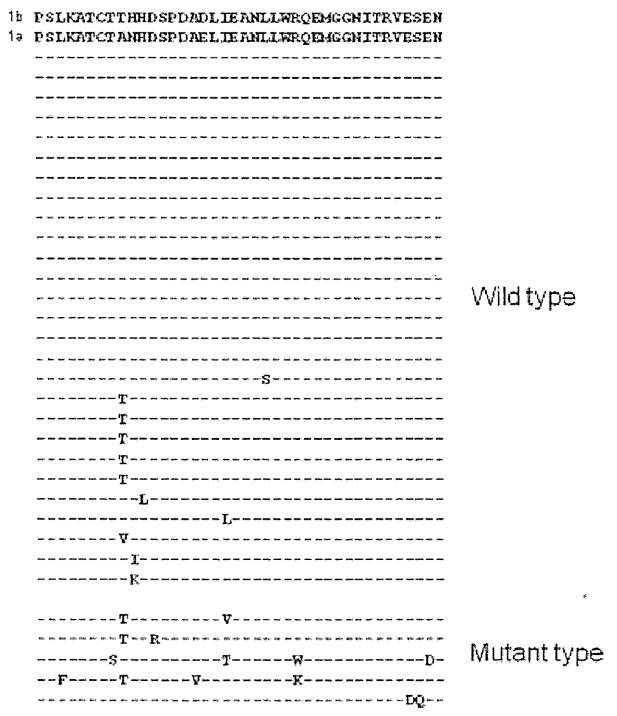

Fig. 1. Alignment of the amino acid sequence for the NS5A-ISDR. In the sequence alignment, dashes indicate amino acids identical to consensus sequence HCV1. Sequences of the HCV1 strain and HCV1 strains with one-nucleotide substitutions were defined as wild-type ISDR, and all other strains were defined as mutant-type ISDR. ISDR, interferon sensitivity-determining region.

TABLE IV. Univariate Analysis: Factors Predictive of Sustained Virologic Response

Factors	Sustained virologic response (n = 11)	Non-sustained virologic response (n = 12)	*P*-value
Age (y.o.)	37.9 ± 10.9	39.8 ± 11.3	0.6958
Gender: male/female	10/1	10/2	0.9999
ALT (IU/L)	78.2 ± 50.8	62.6 ± 68.1	0.5435
AST (IU/L)	51.4.4 ± 29.2	48.8 ± 40.4	0.8616
PLT ($\times 10^4$/mm^3)	19.0 ± 5.4	19.3 ± 5.7	0.8870
HCV RNA level (KIU/ml)	1323.1 ± 1077.3	2567.0 ± 2940.8	0.2481
ISDR: wild/mutant	7/4	12/0	0.0373
IL28B:TT/TG	9/1	4/8	0.0115

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; IL28B, interleukin 28B.

the pathogenesis of HCV genotype 1a infection. However, the HCV core region of genotype 1a is well-conserved and no significant mutations were seen in the core region, which is associated with IFN responsiveness. Several reports have also found that the HCV core region, including positions 70 and 91, of HCV genotype 1a is highly conserved [Alestig et al., 2011; Kumthip et al., 2011]. Mutations in the core region of genotype 1a would be rare, so this region might be unsuitable for routine clinical use, unlike in genotype 1b. However, the number of patients in this study was small, and large studies including from other countries are needed to clarify these issues. The ISDR in the NS5A region of HCV genotype 1b is closely associated with response to IFN therapy. ISDR mutations of genotype 1b are well known to be more important in predicting sustained virological response in Japanese patients than European patients [Hofgärtner et al., 1997; Zeuzem et al., 1997; Nakano et al., 1999; Pascu et al., 2004; Hayashi et al., 2011a]. European studies have failed to detect the specific amino acid substitutions in ISDR of genotype 1a associated with IFN responsiveness [Hofgärtner et al., 1997; Zeuzem et al., 1997]. In this study, sustained virological response was achieved in 36.8% of patients with wild-type ISDR and 100% of patients with mutant-type ($P = 0.0373$). The present analysis showed a close relationship between ISDR of genotype 1a and sustained virological response, as in genotype 1b. Recent investigations in Thailand and Iran have failed to identify the usefulness of ISDR for HCV genotype 1a in predicting sustained virological response [Kumthip et al., 2011; Yahoo et al., 2011]. The high virological response rate and low prevalence of patients with mutations in the ISDR do not favor the use of ISDR analysis in predicting IFN responsiveness [Herion and Hoofnagle, 1997; Yokozaki et al., 2011]. Rates of sustained virological response among these studies were much higher than those in the present study (68.4% and 75% vs. 47.8%). The mean number of mutations in patients who achieved sustained virological response in the studies by Kumthip et al. [2011] and Yahoo et al. [2011], and the present group were 1.4, 1.4, and 1.6, respectively. Differences in sustained virological response and the number of mutations to the ISDR might underpin this discrepancy in the evaluation of ISDR. Although the sample size in the present study was small, the results indicate that ISDR represents a strong indicator of progression to sustained virological response for patients with HCV genotype 1a. Amino acid substitutions in the ISDR of genotype 1a thus also play an important role in predicting sustained virological response in Japanese patients compared to patients from other countries. IL28B polymorphisms such as host genetics, as well as mutations in the HCV genome, contribute to IFN treatment outcomes. Rates of sustained virological response in patients in this study with TT and TG were 69.2% and 11.1%, respectively. The TG allele of the IL28B genotype was significantly associated with poor response to IFN therapy ($P = 0.0115$). SNPs of IL28B would regulate the expression of IFN-stimulated genes and affect IFN responsiveness. IL28B and ISDR thus exert independent effects on IFN responsiveness and both host and viral factors impacting IFN responsiveness would improve the prediction of sustained virological response. Several studies have thus reported that both the SNP of IL28B and mutations in the ISDR were associated with sustained virological response in patients with HCV genotype 1b [Akuta et al., 2011; Hayashi et al., 2011b; Kurosaki et al., 2011]. In the present study of HCV genotype 1a, among the 9 patients who had simultaneously the TG allele for IL28B and wild-type ISDR, only 1 achieved sustained virological response (11.1%). The best-sustained virological response was achieved in patients with mutant-type ISDR and the T allele (100%). The combination of SNPs for IL28B and mutations in ISDR may thus predict response to IFN therapy in patients with HCV genotype 1a as well as genotype 1b. Given the small sample size in this investigation, larger cohorts are needed to confirm the present results. Furthermore, infection with genotype 1a in Japanese patients is rare, making large-scale studies difficult to perform.

In conclusion, the prevalence of HCV genotype 1a is rare in Japan and the majority of cases involve patients with hemophilia. The TG genotype of IL28B is associated with poor response, while mutant-type ISDR is associated with good response to combination therapy with pegylated-IFN-α2b and ribavirin in patients with HCV genotype 1a. Combined use of both IL28B and ISDR could improve the prediction of IFN response.

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Review Article

Can non-invasive assessment of liver fibrosis replace liver biopsy?

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Transient elastgraphy, acoustic radiation force impulse and real-time elastography are the methods with very good or excellent diagnostic accuracy for the assessment of liver fibrosis stage. They do not provide the information on inflammatory activity, steatosis, iron deposition or other findings derived from liver biopsy. Even on account of fibrosis stage, these non-invasive methods do not give us the estimation completely corresponding to that of liver biopsy. However they provide us useful clinical information that liver biopsy has been providing us, such as appropriate time to start antiviral therapy, prediction of response to antiviral therapy, evaluation of effects of antiviral therapy, assessment of natural course of hepatitis and estimation of prognosis of hepatitis. Recently non-invasive methods for assessment of inflammatory activity, steatosis and iron deposition in the liver have been developed. Thus in the near future, non-invasive methods will replace liver biopsy.

Key words: acoustic radiation force impulse, fibrosis stage, inflammatory activity, liver stiffness, real-time elastography, transient elastography

INTRODUCTION

NON-INVASIVE ASSESSMENT OF liver fibrosis has been one of major objectives in the society of hepatologists for a long time. Routine laboratory tests, serum markers of fibrosis[1-7] and apparatuses for measuring liver stiffness (LS) have been tested. The apparatuses include transient elastography (TE),[8,9] acoustic radiation force impulse (ARFI),[10] real-time elastography,[11] and magnetic resonance imaging (MRI).[12]

Liver biopsy is the gold standard for the assessment of fibrosis stage in chronic viral hepatitis. However, liver biopsy is an invasive and expensive procedure, and its accuracy is sometimes questionable because of sampling errors, inadequate specimens and the subjectivity of diagnosis.[13,14]

Infections of hepatitis B virus (HBV) and hepatitis C virus (HCV) are world-wide problems and cause the need of a great number of liver biopsies mainly for assessment of fibrosis stage and inflammatory activity, which sometimes cause serious complications. Thus the replacement of liver biopsies with non-invasive methods is an important subject to be deal with as soon as possible.

In this article, we review the manuscripts that applied non-invasive methods to estimate fibrosis stages for the five different clinical aims in the replacement of liver biopsies. These aims include the determination of appropriate time to start antiviral therapy, prediction of response to antiviral therapy, evaluation of effects of antiviral therapy, assessment of natural course of hepatitis and estimation of prognosis of hepatitis. We will discuss whether non-invasive methods can replace liver biopsies for these aims.

We discuss the three methods that have been often reported; TE, ARFI imaging, and real-time elastography. Algorithm of serum fibrosis markers such as FibroTest[2] will be also described. There have been published a lot of manuscripts on non-invasive methods, and we selected the manuscripts that seem to us to be important in discussing whether non-invasive methods can replace liver biopsies.

Transient elastography measures LS with the use of an apparatus, FibroScan (EchoSens, Paris, France).[8] FibroScan is equipped with a probe including an

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ultrasonic transducer and a vibrator. A vibration of mild amplitude and low frequency is transmitted from the vibrator placed on the body surface toward the liver through the intercostal space. The vibration induces an elastic shear wave that propagates through the liver tissue. The pulse-echo ultrasound acquisitions follow the propagation of the shear wave and determine its velocity. The velocity is directly related to tissue stiffness; the harder the tissue, the faster the shear wave propagates. LS is calculated from velocity and expressed in kilopascal (kPa).

Acoustic radiation force impulse imaging is a radiation force-based imaging method that is provided with conventional B-mode ultrasonography (Siemens Acuson S2000, Siemens AG, Germany).[10] In ARFI imaging, an initial ultrasonic pulse is transmitted at diagnostic intensity levels to obtain a baseline signal for later comparison. A short-duration, high-intensity acoustic pushing pulse is transmitted from the probe, and cause shear wave in the liver. A series of diagnostic intensity pulses are used to quantitate shear wave velocity (Vs; m/s). The velocity of the shear wave depends on LS.

Real-time elastography is an imaging technique that can reveal the physical property of tissue using conventional ultrasound probes; the Hitachi EUB-8500 and EUB-900 machines (Hitachi Medical Systems, Tokyo, Japan).[11] The region of interest is divided up in to 30 000 finite elements before compression. During the compression by the probe or heart beats, the displacement of each element is measured. In hard tissue, the amount of displacement is low, whereas in soft tissue, the amount of displacement is high. The calculation of tissue elasticity distribution is performed in real time, and the results are displayed as color-coded images with the conventional B-mode image in the background. In this way, a large number of summarizing variables were obtained to characterize elastography. The final score was based on 10 summarizing variables selected from them to obtain high reproducibility. The variables selected for the final score differ among the investigators.

APPROPRIATE TIME TO START ANTIVIRAL THERAPY: DIAGNOSIS OF SIGNIFICANT FIBROSIS (F> OR =2)

IN CHRONIC VIRAL hepatitis, the presence of significant fibrosis (F> or =2) indicates the need of antiviral therapies both in chronic hepatitis B and in chronic hepatitis C.[15,16]

A meta-analysis of the performance of TE for staging of liver fibrosis demonstrated that the area under the receiver operating characteristic curve (AUROC) for significant fibrosis ranged 0.68–1.0 among different studies with a mean of 0.84 (95% confidence intervals [CI], 0.82–0.86) and an adjusted AUROC of 0.91 and that the optimal cut-off value for the significant fibrosis suggested from the summary ROC techniques was 7.65 kilopascals (kPa).[17]

We published a review article on the investigations of TE for assessment of fibrosis stages and presented the summary table.[18] Thus we do not show the table in the present article.

Friedrich-Rust *et al.* studied 134 patients with chronic liver diseases and reported that the AUROC for the diagnosis of significant fibrosis of real-time elastography, TE and FibroTest was 0.69, 0.84 and 0.85, respectively.[19]

Koizumi measured LS with real-time tissue elastography in 70 patients with chronic hepatitis C[20]. The elastic ratio (ratio of the value in the intrahepatic venous small vessels divided by the value in the hepatic parenchyma) was calculated. The cut-off value and AUROC for significant fibrosis were 2.73 and 0.89, respectively.

Although real-time elastography is a hopeful non-invasive method, the calculations of elastic value differ among the investigators. Thus we think it is inappropriate to present the summary table.

Friedrich-Rust *et al.* studied 86 patients with chronic viral hepatitis and reported that the AUROC for the diagnosis of significant fibrosis of ARFI, TE, and FibroTest was 0.82, 0.84, and 0.82, respectively.[10] The cut-off values for significant fibrosis of ARFI and TE were 1.37 m/s (sensitivity 68.5%, specificity 92.6%) and 6.3 kPa (sensitivity 83.3%, specificity 74.1%), respectively.

Takahashi *et al*[21] studied 55 patients mainly consisting of people with HCV by ARFI. The AUROC and cut-off value of the Vs for significant fibrosis were 0.94 (95% CI, 0.87–0.99) and 1.34 m/s (sensitivity 91.4%, specificity 80%).

Fierbinteanu-Braticevici[22] studied 74 patients with HCV by ARFI. The AUROC and cut-off value of Vs for significant fibrosis were 0.902 (95% CI, 0.831–0.972, $P < 0.001$) and 1.215 m/s (sensitivity 100%, specificity 71%).

The summary of investigations of ARFI for assessment of significant fibrosis is shown in Table 1.[10,21–29]

Generally the diagnostic accuracy of test with AUROC of 0.7–0.8 was considered as good, that of 0.8–0.9 as very good, and that of 0.9–1.0 as excellent. The diagnostic accuracy of TE, ARFI and real-time elastography for significant fibrosis is very good or excellent. They do not give us the estimation completely corresponding to that of liver biopsy; in our study (AUROC 0.88; sensitivity 81%;

Table 1 Summary of investigations of acoustic radiation force impulse for assessment of liver fibrosis

Author (year) reference	Disease	Number of patients	System of fibrosis staging	Fibrosis stage							
				F> or =1		F> or =2		F> or =3		F> or =4	
				Cut-off value (m/s)	AUROC	Cut-off value (m/s)	AUROC	Cut-off value (m/s)	AUROC	Cut-off value (m/s)	AUROC
Friedrich-Rust (2009)[10]	Chronic viral hepatitis	86	Metavir			1.37	0.82	1.45	0.91	1.75	0.91
Lupsor (2009)[23]	HCV	112	Metavir	1.19	0.725	1.34	0.869	1.61	0.9	2	0.936
Takahashi (2009)[21]	Chronic liver disease	55	Metavir			1.34	0.94	1.44	0.94	1.8	0.96
Fierbinteanu-Braticevici (2009)[22]	HCV	74	Metavir	1.185		1.215	0.902	1.54	0.993	1.94	0.993
Sporea (2011)[27]	Chronic liver disease	76	Metavir	1.4	0.747					1.78	0.951
Gigurevic (2011)[28]	Chronic liver disease	38	Ishak							1.86	0.99
Sporea (2010)[24]	Chronic viral hepatitis	71	Metavir			1.33	0.649			1.8	0.868
Toshima (2011)[25]	Chronic liver disease	79	Scheuer	1.45	0.81	1.52	0.81	1.69	0.85	1.79	0.87
Piscaglie (2011)[29]	Chronic liver disease	90								1.75	0.941
Ebinuma (2011)[26]	Chronic viral hepatitis	59	Metavir			1.4	0.905	1.53	0.923	1.88	0.854

AUROC, area under the receiver operating characteristic curve; HCV, hepatitis C virus.

specificity 80%), 17 of 42 patients with biopsy proven F2 (40%) had LS by TE corresponding to F0-1.[30] However, they are still useful for determining the indication of antiviral therapies, if we use them in combination with laboratory tests and other clinical data. The combination of TE and biomarkers is being studied to improve the diagnostic accuracy of significant fibrosis.[30,31]

PREDICTION OF RESPONSE TO ANTIVIRAL THERAPY

FIBROSIS STAGE IS an important predictor for response to combination therapy of pegylated interferon (PEG-IFN) and ribavirin for chronic hepatitis C. Hayashi *et al.* reported that the factors related to sustained virological response (SVR) on multivariate analysis were single nucleotide polymorphism (SNP) of interleukin 28B (IL28B) ($P = 0.0001$), fibrosis ($P = 0.0111$) and mutations in the core region70 ($P = 0.0267$) and IFN sensitivity determining region (ISDR) of HCV genome ($P = 0.0408$).[32]

Poynard *et al.* studied the predictive factors for SVR in 1459 patients with chronic hepatitis C retreated with PEG-IFN alfa-2b plus weight-based ribavirin. Uni- (UV) and multi-variable (MV) analyses were performed. Five baseline factors were associated ($P < 0.001$) with SVR in UV and MV analyses (odds ratio: UV/MV): fibrosis stage estimated using FibroTest (4.5/5.9) or biopsy (1.5/1.6), genotype 2/3 (4.5/5.1), viral load (1.5/1.3), prior relapse (1.6/1.6), previous treatment with non-PEG-IFN (2.6/2.0). Poynard *et al.* concluded that FibroTest at baseline is a possible non-invasive alternative to biopsy for the prediction of SVR, in patients with previous failures and advanced fibrosis, retreated with PEG-IFN alfa-2b and ribavirin.[33]

We have studied the predictive factors for SVR in 88 patients with chronic hepatitis C genotype 1 treated with combination of IFN and ribavirin and found that gender ($\beta = 1.6$, $P = 0.0012$) and LS by TE ($\beta = -0.1$, $P = 0.0214$) are independent predictive factors by multivariate analysis (manuscript in preparation).

Thus FibroTest and LS by TE can substitute liver biopsy for the purpose of predicting response to antiviral therapy in chronic hepatitis C.

EVALUATION OF EFFECTS OF ANTIVIRAL THERAPY

THE OUTCOME OF antiviral therapy should be assessed not only by ALT levels or viral loads but

also by the alleviation of fibrosis stage both in chronic hepatitis B and in chronic hepatitis C.

Ogawa *et al.* studied 145 HCV infected patients treated with PEG-IFN plus ribavirin by TE[34]. LS were significantly decreased in SVR patients (the mean rate of change; −16.2%, −32.2% and −43.5%) in comparison with non-SVR patients (−7.2%, −2.1% and +17.3%) at the end of treatment (EOT) ($P = 0.0127$), and 48 weeks ($P < 0.0001$) and 96 weeks ($P < 0.0001$) after EOT. Among non-SVR patients, LS were significantly decreased in patients with biochemical response (BR) (−17.9%, −30.0% and −27.1%) in comparison with non-BR (−4.1%, +6.4% and +30.6%) at EOT ($P = 0.0270$), and 48 weeks ($P < 0.0001$) and 96 weeks ($P < 0.0001$) after EOT.

Arima *et al.* measured LS by TE before treatment, at EOT, one year and 2 years after EOT in 145 patients with chronic hepatitis C treated by IFNs with or without ribavirin.[35] In 93 patients with SVR and 28 relapsers, LS significantly decreased at EOT (median, 5.4 [interquartile range, 4.0–8.6] kPa, $P < 0.0001$ and 6.8 [4.5–8.9] kPa, $P = 0.0023$) and one year after EOT (5.3 [4.2–7.0] kPa, $P < 0.0001$ and 6.8 [4.5–9.3] kPa, $P = 0.0204$) compared with baseline (8.0 [5.0–11.9] kPa and 10.6 [7.0–16.6] kPa). In SVR patients, LS significantly decreased 2 years after EOT (5.3 [4.1–6.3] kPa) compared with baseline ($P < 0.0001$) and LS at EOT ($P = 0.0034$). In 24 patients with non virological response (NVR), LS at EOT, one year after EOT, and 2 years after EOT did not significantly differ from pretreatment values.

Arima *et al.* proposed the use of deduced fibrosis stage from LS based on cut-off values for fibrosis stage. The use of deduced fibrosis stage enables evaluation of the degrees of changes of LS. 2-point or greater reduction of deduced stage was observed in 78% (29/37) of SVR patients, 59% (10/17) of relapsers and 15% (2/13) of NVR patients. A 2-point or greater decrease of deduced fibrosis stage were associated with milder baseline fibrosis stage, lower hyaluronic acid levels, longer IFN treatment, virological response of SVR or relapse and higher ALT levels.

Thus, we can assess not only the alleviation of fibrosis but also the factors that affect the alleviation of fibrosis by measuring LS in chronic hepatitis C.

Wang *et al.* studied LS by TE in 144 patients receiving IFN-based therapy, including 95 SVR patients and 49 non-SVR patients.[36] There was a significant decrease of LS among SVR patients (median, 0.6; $P < 0.001$). non-SVR patients showed an increase of LS (median, 0.8; $P = 0.557$). For SVR patients, a high initial LS was the predictive factor of a rapid reduction of LS values. However, advanced fibrosis stage before therapy, higher body mass index (BMI) and longer time remission were predictive factors for slow reduction of LS values.

Osakabe *et al.* measured LS by TE in 29 HBV-infected patients treated with nucleotide or nucleoside analogs and assessed the changes of LS.[37] By antiviral therapy, LS significantly reduced from 12.9 (6.2–17.9) kPa to 6.6 (4.4–10.3) kPa in the interval of 512 (366–728) days ($P < 0.0001$). Eleven of 19 (58%) patients with baseline fibrosis stages of F3-4 deduced from LS had 2-point or greater reduction of deduced stage at last LS measurement. The change ratio of hyaluronic acid ($P = 0.0390$) was associated with a 2-point or greater reduction.

Enomoto *et al.* studied LS by TE in 50 patients with chronic hepatitis B virus infection.[38] LS of the patients with entecavir significantly decreased from 11.2 kPa (7.0–15.2) to 7.8 kPa (5.1–11.9; $P = 0.0090$) during 12 months of treatment.

It is difficult to repeat liver biopsies after or during antiviral therapy to assess its effect. Since there is the heterogeneity of the effect of treatment, it is important to know who is a good responder or not and investigate the factors affecting the effect of therapy. Non-invasive measurement of LS can be done repeatedly and provide the information of effect of antiviral therapy.

The results of TE were not confirmed by the results of liver biopsies in the articles reviewed. The absence of comparison with biopsies is the limitations of these studies.

ASSESSMENT OF NATURAL COURSE OF VIRAL HEPATITIS

ARIMA *ET AL.* STUDIED 35 patients with chronic HCV infection without IFN treatment and reported that LS at 2nd measurement (12.2 [6.3–16.8] kPa) did not differ significantly from LS at 1st measurement (10.5 [5.8–15.3] kPa) in the interval of 656 (360–922) days.[35]

Osakabe *et al.* reported that, in 52 HBV-infected patients without antiviral therapy, LS tended to increase from 6.1 (3.9–8.5) kPa to 6.3 (4.4–9.7) kPa in the interval of 422 (358–709) days ($P = 0.0682$).[37] Without antiviral therapy, 11 of 50 (22%) patients with deduced fibrosis stages of F0-3 at 1st measurement had an increase of deduced stage, while 8 of 20 (40%) patients with deduced fibrosis stages of F2-4 at 1st measurement had a reduction of deduced stage. The factor associated with an increase of deduced fibrosis stage was lower baseline albumin levels ($P = 0.0092$).

The reason why the significant increase of LS was not detected in the natural course in these reports is

probably attributed to the fact that the subjects of the studies are the patients who had mild disease and needed no antiviral therapy. TE would be a useful tool to detect the patients with progressive fibrosis for the physicians in the follow-up of the patients with chronic viral hepatitis.

The results of TE were not confirmed by the results of liver biopsies in the articles reviewed. The absence of comparison with biopsies is the limitations of these studies.

ESTIMATION OF PROGNOSIS OF HEPATITIS

THE RISK OF hepatocellular carcinoma (HCC) or bleeding from esophageal varices is high in patients with advanced fibrosis.[39,40] Thus it is important to detect advanced fibrosis early and start the search for HCC and varices in order to treat them in early stage or before bleeding.

A meta-analysis of performance of TE for fibrosis staging demonstrated that the mean AUROC for cirrhosis was 0.94 (95% CI, 0.93–0.95) and an adjusted AUROC of 0.99 and that the optimal cut-off value for cirrhosis suggested from the summary ROC techniques was 13.01 kPa.[17]

Piscaglia *et al.* studied 90 patients with chronic liver disease with ARFI.[29] The AUROC for the diagnosis of cirrhosis was 0.941 with 1.75 m/s as the optimal cut-off (sensitivity 93.0%; specificity 85.1%).

Lupsor *et al.* studied 112 patients with chronic hepatitis C with ARFI.[23] The AUROC for the diagnosis of cirrhosis was 0.936 with 2 m/s as the optimal cut-off (sensitivity 80.0%; specificity 95.45%).

Sporea *et al.* studied 71 patients with chronic liver diseases with ARFI.[24] The AUROC for the diagnosis of cirrhosis was 0.868 with 1.8 m/s as the optimal cut-off (sensitivity 100%; specificity 77%).

Toshima *et al.* studied 79 patients with chronic liver diseases with ARFI.[25] The AUROC for the diagnosis of cirrhosis was 0.87 with 1.79 m/s as the optimal cut-off (sensitivity 86%; specificity 79%).

Ebinuma *et al.* studied 59 patients with chronic viral hepatitis with ARFI.[26] The AUROC for the diagnosis of cirrhosis was 0.854 with 1.88 m/s as the optimal cut-off (likelihood ratio 4.55).

The summary of investigations of ARFI for assessment of cirrhosis is shown in Table 1.[10,21–29]

Friedrich-Rust *et al.* studied 79 patients with chronic viral hepatitis with real-time elastography.[11] The cut-off value of elastic ratio and AUROC for cirrhosis was 111.75 and 0.69, respectively (sensitivity 29.2%; specificity 90.7%).

Koizumi measured LS with real-time tissue elastography in 70 patients with chronic hepatitis C[20]. The cut-off value of elastic ratio and AUROC for cirrhosis were 3.93 and 0.95, respectively (sensitivity 90.9%; specificity 91.5%).

Stefanescu et al. compared the performance of common serum fibrosis scores and TE in diagnosing esophageal varices in 231 cirrhosis patients.[41] The Lok Score[42] was the best among all the serum scores for diagnosing the varices; cut-off value for large varices is 0.8 (positive predictive value 45.5%, negative predictive value 86.4% and diagnostic accuracy 67.72%). The cut-off value of LS for large varices is 30.8 kPa (positive predictive value 47.3%, negative predictive value 81% and diagnostic accuracy 68.32%). Using both tests simultaneously, the presence of large varices was predicted with a diagnostic accuracy of 78.12%, obtaining an increment in negative predictive value and negative likelihood ratio up to 93.67% and 0.21, respectively.

Jung *et al.* investigated the usefulness of LS by TE as a predictor of HCC development in 1130 patients with chronic HBV infection.[43] During the follow-up period (median, 30.7 months; range, 24.0–50.9 months), HCC developed in 57 patients (2.0% per 1 person-year). The 1-, 2-, and 3-year cumulative incidence rates of HCC were 0.80%, 3.26%, and 5.98%, respectively. On multivariate analysis, together with old age, male sex, heavy alcohol consumption (>80 g/day), serum albumin, and hepatitis B e antigen positivity, patients with a higher LS (>8 kPa) were at a significantly greater risk of HCC development, with the following hazard ratios: 3.07 (95% confidence interval [CI], 1.01–9.31; $P = 0.047$) for LS 8.1–13 kPa; 4.68 (95% CI, 1.40–15.64; $P = 0.012$) for LS 13.1–18 kPa; 5.55 (95% CI, 1.53–20.04; $P = 0.009$) for LS 18.1–23 kPa; and 6.60 (95% CI, 1.83–23.84; $P = 0.004$) for LS > 23 kPa.

Masuzaki *et al.* investigated the relationship between LS and HCC presence in the cross-sectional study.[44] LS was measured in chronic hepatitis C patients (85 with HCC and 180 without) by TE. Multivariate analysis showed that HCC presence was significantly associated with LS ($P < 0.0001$) along with age, male, and α-fetoprotein concentration. AUROC was 0.805, 0.741, 0.714, 0.673, 0.670, and 0.654 for LS, α-fetoprotein, albumin, prothrombin activity, aspartate aminotransferase (AST)-platelet ratio index, and platelet count, respectively. Stratum-specific likelihood ratio for HCC presence by LS was 0.22 (95% CI: 0.11-0.42) in

<10 kPa, 0.73 (0.39 to 1.39) in 10.1 to 15 kPa, 1.30 (0.80 to 2.12) in 15.1 to 25 kPa, and 5.0 (2.96 to 8.47) in >25 kPa.

Masuzaki *et al.* investigated the relationship between baseline LS and HCC development prospectively among 866 patients with chronic hepatitis C.[45] During the follow-up period (mean, 3.0 years), HCC developed in 77 patients (2.9% per 1 person-year). The cumulative incidence rates of HCC at 1, 2, and 3 years were 2.4%, 6.0%, and 8.9%, respectively. Adjusting for other significant factors for HCC development, patients with higher LS were revealed to be at a significantly higher risk, with a hazard ratio, as compared to LS< or =10 kPa, of 16.7 (95% CI, 3.71–75.2; $P < 0.001$) when LS 10.1–15 kPa, 20.9 (95% CI, 4.43–98.8; $P < 0.001$) when LS 15.1–20 kPa, 25.6 (95% CI, 5.21–126.1; $P < 0.001$) when LS 20.1–25 kPa, and 45.5 (95% CI, 9.75–212.3; $P < 0.001$) when LS > 25 kPa.

Thus TE, real-time elastography and ARFI are useful for diagnosis of cirrhosis and prediction of development of varices or HCC.

CAN LIVER STIFFNESS REPLACE LIVER BIOPSY?

TRANSIENT ELASTOGRAPHY, ARFI and real-time elastography are the methods with very good or excellent diagnostic accuracy for the assessment of liver fibrosis stage. They do not provide information on inflammatory activity, steatosis, iron deposition or other findings in liver biopsy. Even on account of fibrosis stage, these non-invasive methods do not give us the estimation completely corresponding to that of liver biopsy. In addition, the values of LS might be affected by factors other than fibrosis stage, for example, inflammatory activity[9,18] and intrahepatic pressure.[46] However they provide us useful clinical information, which liver biopsy has been providing us as described in the present article, such as appropriate time to start antiviral therapy, prediction of response to antiviral therapy, evaluation of effects of antiviral therapy, assessment of natural course of hepatitis and estimation of prognosis of hepatitis. Recently non-invasive methods for assessment of inflammatory activity,[47] steatosis[48,49] and iron deposition[50] in the liver have been developed. Such as ActiTest,[47] SteatoTest,[49] and MR imaging for quantification of fat[48] and iron contents[50] in liver provide the information other than fibrosis derived from liver biopsy. Thus in the near future, non-invasive methods will replace liver biopsy.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Reduction of liver stiffness by antiviral therapy in chronic hepatitis B

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Abstract
Background Liver stiffness (LS) has been reported to correlate with fibrosis stage (F). The correlation between LS and fibrosis stage and the reduction of LS by antiviral therapy were examined in patients with hepatitis B infection.
Methods LS was measured by FibroScan in 212 patients infected with hepatitis B virus. Liver biopsies were done in 51 patients. Changes of LS were assessed in 29 patients treated with nucleotide or nucleoside analogs and 52 patients without antiviral therapy.
Results LS was significantly correlated with fibrosis stage ($\rho = 0.686$, $P < 0.0001$). The optimal cut-off values of LS were 7.1 kPa for F $\geq$ 2, 10.7 kPa for F $\geq$ 3, and 16.0 kPa for F4. LS was significantly reduced by antiviral therapy, from 12.9 (range 6.2–17.9) kPa to 6.6 (4.4–10.3) kPa measured at an interval of 512 (range 366–728) days ($P < 0.0001$). Eleven of 19 (58%) patients with baseline fibrosis stages of F3-4 deduced from LS had 2-point or greater reductions of deduced stage at the last LS measurement. The change ratio of hyaluronic acid ($P = 0.0390$) was associated with a 2-point or greater reduction of deduced fibrosis stage. Without antiviral therapy, LS tended to increase, increasing from 6.1 (range 3.9–8.5) kPa to 6.3 (range 4.4–9.7) kPa at an interval of 422 (range 358–709) days ($P = 0.0682$).
Conclusions LS was significantly correlated with fibrosis stage in patients with chronic hepatitis B. The reduction of LS by antiviral therapy was significantly correlated with the reduction of hyaluronic acid. Thus, we conclude that LS can be useful to assess the progression and regression of liver fibrosis stage noninvasively.

Keywords Hepatitis B · Antiviral therapy · Liver stiffness · Transient elastography · Fibrosis stage

Abbreviations

LS	Liver stiffness
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
TE	Transient elastography
kPa	Kilopascals
ROC	Receiver operating characteristics
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
APRI	Aminotransferase-to-platelet ratio index

Introduction

Chronic infection with hepatitis B virus (HBV) currently affects about 400 million people, with particularly high prevalence in developing countries, and it is estimated that worldwide more than 200,000 and more than 300,000

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chronic HBV carriers die each year from cirrhosis and hepatocellular carcinoma (HCC), respectively. The most important predictors of cirrhosis or HCC in patients with chronic hepatitis B are persistently elevated HBV DNA and alanine aminotransferase (ALT) levels [1, 2]. It has been estimated that up to 60% of HBV-related HCC occurs in patients with cirrhosis, while almost all HCV-related HCC occurs in the setting of cirrhosis [3]. Thus, the diagnosis of fibrosis stages is important not only for assessing prognosis and the need for antiviral therapy but also for identifying patients with cirrhosis who are likely to develop HCC.

Liver biopsy is currently considered the gold standard for assessing fibrosis stage in chronic liver disease. However, it is an invasive procedure, with rare but potentially life-threatening complications. In addition, the accuracy of liver biopsy in assessing fibrosis has limitations because of sampling errors and interobserver variability [4–6].

Over the past several years, many researchers have shown an interest in the estimation of liver fibrosis in liver diseases by transient elastography. Transient elastography is a rapid, noninvasive, and reproducible method for measuring liver stiffness (LS). The LS measurement can be performed in about 95% of patients but is problematic in those with ascites or a body mass index above 28 kg/m^2. However the intra- and interoperator reproducibility of LS measurement has been proven by a previous study [7].

LS has been reported to correlate with the stage of liver fibrosis in various liver diseases [8–20]. In addition, serial measurements of LS were shown to be useful to follow up patients with liver disease [8, 9]. However, there have been only a few studies of LS in patients with HBV infection [21–25].

In the present study, we evaluated the correlation of LS with biological parameters in 212 patients with chronic hepatitis B, and we evaluated the correlation of LS with the fibrosis stage in 51 patients. In addition, we evaluated changes in LS in 81 patients, 29 of whom received antiviral therapy.

Patients and methods

Patients

Two hundred and twelve patients with chronic HBV infection diagnosed consecutively at Fujita Health University Hospital from November 2005 to December 2009 were examined for LS. We evaluated the correlation of LS with biological, serological, and virological parameters in these 212 patients who had not received antiviral therapy. Liver biopsy was performed in 51 patients. In 81 patients, LS was measured more than twice. Twenty nine of the 81 patients were subsequently treated with nucleotide or nucleoside analogs and 52 patients did not receive antiviral therapy (Fig. 1). Eight patients were treated with lamivudine and 21 with entecavir (Table 1).

The biochemical, serological, and virological examinations were performed within 2 days of the LS measurements. Aminotransferase-to-platelet ratio index (APRI) values were calculated using the formula: aspartate aminotransferase (AST) [IU/L]/platelets [10^9/L] × 100 [26]. FIB-4 values were calculated using the formula: age (years) × AST [IU/L]/(platelets [10^9/L] × (ALT [IU/L])$^{1/2}$) [27].

According to the guidelines for the treatment of chronic hepatitis and cirrhosis due to HBV infection, patients younger than 35 years did not receive antiviral therapy, except for those who were hepatitis B e antigen (HBeAg)-negative with a platelet count of less than 150 × 10^3/μL or fibrosis stage F2 or higher. Patients aged 35 years or older with ALT of ≥ 31 IU/L received antiviral therapy [28].

Patients were recommended to have a liver biopsy before making the decision about starting antiviral therapy,

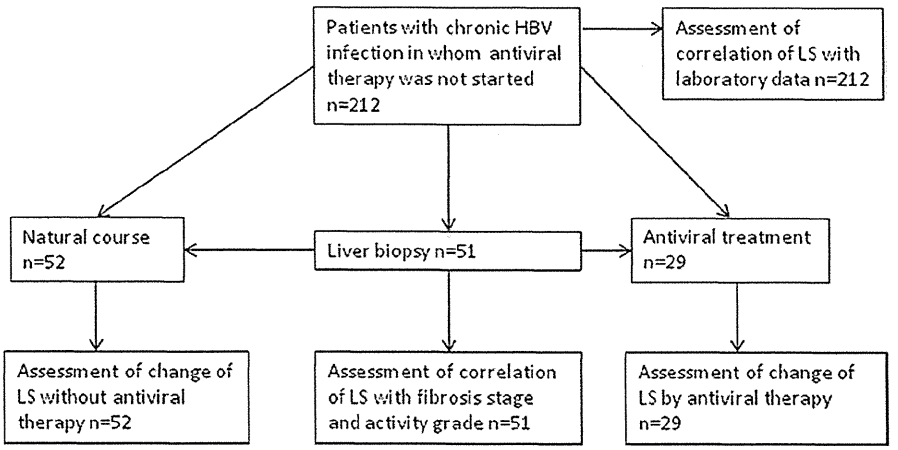

Fig. 1 Flow chart of the study patients. *HBV* hepatitis B virus, *LS* liver stiffness

Table 1 Clinical and biological characteristics of the patients

	Patients for baseline study of correlation of liver stiffness and laboratory data	Subjects of follow-up study		*P* value
		Antiviral therapy	Without treatment	
Number of patients	212	29	52	
Age (years)	51 (39–60)	49 (41–62)	45 (31–59)	0.0438
Gender (female/male)	78/134	11/18	20/32	NS
AST (IU/L)	29.0 (21.0–57.0)	51.0 (39.0–93.5)	28.0 (21.0–56.8)	0.0011
ALT (IU/L)	34.0 (20.3–80.0)	66.0 (33.5–116.0)	39.5 (19.3–84.5)	0.0294
Total bilirubin (mg/dL)	0.9 (0.7–1.2)	1.0 (0.7–1.7)	1.0 (0.8–1.2)	NS
Total protein (g/dL)	7.5 (7.1–7.9)	7.4 (7.2–7.9)	7.6 (7.3–8.0)	NS
Albumin (g/dL)	4.4 (4.0–4.6)	4.3 (3.8–4.5)	4.4 (4.1–4.6)	NS
γ-Globulin (%)	17.7 (15.2–20.9)	21.4 (15.3–26.8)	16.9 (14.0–19.3)	NS
Platelet count ($\times 10^4/\mu L$)	17.6 (13.5–20.8)	12.2 (9.1–17.0)	18.8 (14.3–24.1)	<0.0001
Prothrombin time (%)	92 (81–100)	86 (77–97)	89 (82–98)	NS
Hyaluronic acid (ng/mL)	33.0 (20.5–98.0)	133.0 (34.3–170.8)	29.0 (21.0–83.0)	0.0029
APRI	0.54 (0.33–1.09)	1.16 (0.68–2.31)	0.45 (0.34–0.95)	<0.0001
FIB-4	1.57 (1.00–2.43)	2.48 (1.43–5.25)	1.35 (0.80–1.80)	<0.0001
HBeAg (+/−)	69/109	17/10	24/19	NS
HBV DNA (log copy/mL)	4.6 (3.0–7.0)	6.7 (4.1–7.2)	4.5 (3.1–7.6)	NS
HBV genotype (A/B/C/F)	2/9/142/1	0/1/20/0	0/1/28/0	NS
Antiviral therapy (LAM/ETV)	8/21	8/21	–	–
Liver stiffness (kPa)	6.1 (4.4–9.5)	12.9 (6.2–17.9)	6.1 (3.9–8.5)	<0.0001

Values are medians (interquartile ranges)

P value of Mann–Whitney *U*-test between patients at the beginning of antiviral therapy and those without treatment

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *LAM* lamivudine, *ETV* entecavir, *APRI* aminotransferase-to-platelet ratio index, *NS* not significant, *HBeAg* hepatitis B e antigen

Differences in proportions of patients according to gender, HBe Ag, and hepatitis B virus (HBV) genotype were assessed by χ^2 test between patients at the beginning of antiviral therapy and those without treatment

to assess the diagnosis and prognosis of hepatitis and to confirm the necessity for antiviral therapy.

The study was performed in accordance with the principles of good clinical practice, the principles of the Declaration of Helsinki and its appendices, and local and national laws. Approval for the present study was obtained from the review board of Fujita Health University.

Liver stiffness measurement

LS measurement by transient elastography was performed with a FibroScan® (EchoSens, Paris, France).The FibroScan® is equipped with a probe including an ultrasonic transducer and a vibrator. A vibration of mild amplitude and low frequency is transmitted from the vibrator placed on the body surface toward the liver through the intercostal space. The vibration induces an elastic shear wave that propagates through the liver tissue. Then pulse-echo ultrasound acquisitions follow the propagation of the shear wave and determine its velocity. The velocity is directly related to tissue stiffness; the harder the tissue, the faster the shear wave propagates. LS is calculated from the velocity and is expressed in kilopascals (kPa). LS measurement was performed after an overnight fast. Ten successful acquisitions were performed for each measurement, and the median value was adopted as representative of LS. LS was measured within a month of liver biopsy.

The procedures were performed by two investigators (T.N. and H.S.) who were blind to the clinical, serological, and histological data. These two investigators have 15 and 10 years' experience, respectively, in ultrasound diagnosis and had carried out more than 30 LS measurements before the present study. The agreement rate for LS values by the two investigators was assessed in 10 patients; as well, LS values measured on two different occasions by the same investigator were assessed in these 10 patients. LS values measured by the two investigators did not significantly differ and the coefficient of variation was 5.3% (range 2 to 9%). LS values measured on two different occasions by the same investigator did not significantly differ and the coefficient of variation was 4.6% (range 2 to 7%).

In our follow-up study, LS was measured at pretreatment or the beginning of the study, and at 1 year (range 7 to 18 months), 2 years (range 19 to 30 months), and 3 years after the beginning of the treatment or the study (range 31 to 42 months).

Liver biopsy

Liver biopsies were done in 51 patients before antiviral treatment was initiated or before the start of the study. Liver biopsy was performed using a 14G disposable Tru-cut needle (Tru-Core Biopsy Instrument, Medical Device Technologies, Gainesville, FL) under ultrasonographic guidance.

Liver specimens of at least 1.5-cm length with more than 8 portal tracts were assessed. All biopsy specimens were analyzed by two hepatologists (K.Y. and N.K.) of 30 and 15 years' experience, respectively. There were 5 and 4 specimens where the stage and grade, respectively, evaluated by the 2 hepatologists differed, and the higher stage and grade were adopted. Fibrosis was staged as: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Inflammatory activity was graded as: A0, none; A1, mild; A2, moderate; and A3, severe activity.

Statistical analysis

The correlation between LS and serum biological parameters and fibrosis stages was estimated by the Spearman's rank correlation test. The χ^2 test was used for categorical variables and Fisher's test was used where appropriate. Differences of LS between fibrosis stages were estimated by the Tukey–Kramer test.

The significance of changes in LS values at two points were compared by the Wilcoxon signed-rank test. Analyses of unpaired data were evaluated by the Mann–Whitney *U*-test.

Data values are expressed as medians and interquartile ranges.

The change ratio of a value was calculated by the formula below:

(Value after treatment)/(pretreatment value) × 100 − 100.

The change ratio of a value in patients without antiviral therapy was calculated by the formula below:

(Value at end of observation)/(baseline value) × 100 − 100.

The diagnostic performance of LS was determined in terms of sensitivity, specificity, positive and negative predictive values, diagnostic accuracy, and area under receiver operating characteristic curve (AUROC). Optimal cut-off values for fibrosis stages were determined at the maximum total of sensitivity and specificity.

Statistical analysis was performed with JMP® (SAS Institute, Cary, NC, USA).

Results

Correlation of liver stiffness with biological parameters

LS values were significantly correlated with AST ($\rho = 0.536$, $P < 0.0001$), ALT ($\rho = 0.493$, $P < 0.0001$), total bilirubin ($\rho = 0.248$, $P < 0.0001$), albumin ($\rho = -0.413$, $P < 0.0001$), γ-globulin ($\rho = 0.466$, $P < 0.0001$), platelet count ($\rho = -0.381$, $P < 0.0001$), prothrombin time ($\rho = -0.540$, $P < 0.0001$), HBV DNA ($\rho = 0.315$, $P < 0.0001$), hyaluronic acid ($\rho = 0.578$, $P < 0.0001$), APRI ($\rho = 0.601$, $P < 0.0001$), and FIB-4 ($\rho = 0.390$, $P < 0.0001$) (Table 2). Male gender and HBeAg positivity were associated with a higher LS ($P = 0.0041$ and $P = 0.0003$).

AST ($P = 0.0334$), prothrombin time ($P = 0.0210$), and hyaluronic acid ($P = 0.0381$) were selected as factors independently associated with LS by multiple regression analysis (Table 2).

Liver stiffness and fibrosis stage in the liver biopsy specimens

The liver biopsies of 51 patients were assessed by the METAVIR system. Fibrosis stage was F0 in 2 patients, F1 in 4, F2 in 18, F3 in 13, and F4 in 14. LS was 5.8 (4.9–6.4) kPa for F0-1, 7.0 (5.5–9.5) kPa for F2, 9.5 (6.7–12.5) kPa for F3, and 17.5 (15.3–19.7) kPa for F4 (Fig. 2a). LS significantly differed between F0-1 and F4 ($P < 0.0001$), between F2 and F4 ($P < 0.0001$), and between F3 and F4 ($P = 0.0001$). LS was significantly correlated with fibrosis stage ($\rho = 0.689$, $P < 0.0001$)

The inflammatory grade was A0 in 2 patients, A1 in 12, A2 in 28, and A3 in 9. LS was 7.0 (5.3–10.1) kPa for A0-1, 9.5 (6.2–16.4) kPa for A2, and 14.5 (8.8–17.7) kPa for A3 (Fig. 2b). LS significantly differed between A0-1 and A3 ($P = 0.033$). LS was significantly correlated with the inflammatory grade ($\rho = 0.359$, $P = 0.0098$).

ROC analysis was done to assess the diagnostic value of LS for different fibrosis stages. AUROC values were 0.844 for F ≥ 2, 0.839 for F ≥ 3, and 0.930 for F4. Based on the ROC curves, the optimal discriminant cut-off values were determined at the maximum total of sensitivity and specificity. The cut-off values were 7.1 kPa for F ≥ 2, 10.7 kPa for F ≥ 3, and 16.0 kPa for F4 (Table 3).

Table 2 Factors correlated with liver stiffness in all patients as assessed by Spearman's rank correlation test and multiple regression analysis

	Spearman's rank correlation test		Multiple regression analysis	
	ρ	P value	β	P value
Age (years)	−0.005	NS	–	–
Gender[a]		0.0041	–	–
Male	6.6 (4.7–12.0)			
Female	5.4 (4.0–6.9)			
AST (IU/L)	0.536	<0.0001	−0.856	0.0334
ALT (IU/L)	0.493	<0.0001	0.407	NS
Total bilirubin (mg/dL)	0.248	<0.0001	-0.106	NS
Total protein (g/dL)	−0.096	NS	–	–
Albumin (g/dL)	−0.413	<0.0001	−0.005	NS
γ-Globulin (%)	0.466	<0.0001	0.025	NS
Platelet count ($\times 10^4/\mu L$)	−0.381	<0.0001	0.011	NS
Prothrombin time (%)	−0.540	<0.0001	−0.271	0.0210
HBeAg (+ vs. −)*		0.0003	–	–
Positive	7.2 (5.8–13.7)			
Negative	5.6 (4.1–8.1)			
HBV DNA (log copy/mL)	0.315	<0.0001	0.037	NS
Hyaluronic acid (ng/mL)	0.578	<0.0001	0.299	0.0381
APRI	0.601	<0.0001	0.578	NS
FIB-4	0.390	<0.0001	0.331	NS

Values are medians (interquartile ranges)

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *APRI* aminotransferase-to-platelet ratio index, *NS* not significant

[a] Mann–Whitney *U*-test

Changes in liver stiffness and biochemical and serological parameters

In the patients with antiviral therapy, LS values at pretreatment and at 1, 2, and 3 years after the beginning of treatment were 12.9 (6.2–17.9) kPa, 7.5 (5.4–11.7) kPa, 6.5 (5.1–10.6) kPa, and 4.7 (3.1–7.9) kPa, respectively.

LS was significantly decreased at 1 year ($P < 0.0001$), 2 years ($P = 0.0001$), and 3 years after the beginning of treatment ($P = 0.0060$) compared with LS at pretreatment. LS was significantly decreased at 2 years ($P = 0.0210$) compared with that at 1 year after the beginning of treatment (Fig. 3a).

LS was 12.9 (6.2–17.9) kPa at pretreatment and was significantly reduced, to 6.6 (4.4–10.3) kPa at the last measurement ($P < 0.0001$). The change ratio of LS was −37.5 (−57.0 to −19.0) %. The interval between the pretreatment measurement and the last measurement of LS was 512 (366–728) days.

In the patients without antiviral treatment, LS values at the 1st measurement and at 1, 2, and 3 years after the beginning of the study were 6.1 (3.9–8.5) kPa, 5.4 (3.7–8.7) kPa, 6.3 (5.2–9.8) kPa, and 7.6 (4.1–11.4) kPa, respectively (Fig. 3b). No significant difference was observed between any of these values. In these patients, LS tended to increase, increasing from 6.1 (3.9–8.5) kPa at the 1st measurement to 6.3 (4.4–9.7) kPa at the last measurement ($P = 0.0682$). The change ratio of LS was 8.7 (−16.6 to 43.7) %. The interval between the 1st measurement and the last measurement was 422 (358–709) days.

The change ratio of LS in the patients with antiviral therapy was significantly higher than that in the patients without antiviral therapy ($P < 0.0001$). The intervals between the 1st measurement and the last measurement did not differ significantly between these two patient groups ($P = 0.5721$).

AST levels were significantly lower at 1 year, at 2 years, and at 3 years after the beginning of treatment than at pretreatment ($P = 0.0002$, $P = 0.0039$, and $P = 0.0313$) (Table 4). The AST level was significantly lower at 2 years after the beginning of treatment than at 1 year after the beginning of treatment ($P = 0.0024$). ALT levels were significantly lower at 1 year, at 2 years, and at 3 years after the beginning of treatment than at pretreatment ($P = 0.0001$, $P = 0.0078$, and $P = 0.0313$). The ALT level was significantly lower at 2 years after the beginning of treatment than at 1 year after the beginning of treatment ($P = 0.0081$). The platelet count was significantly higher at

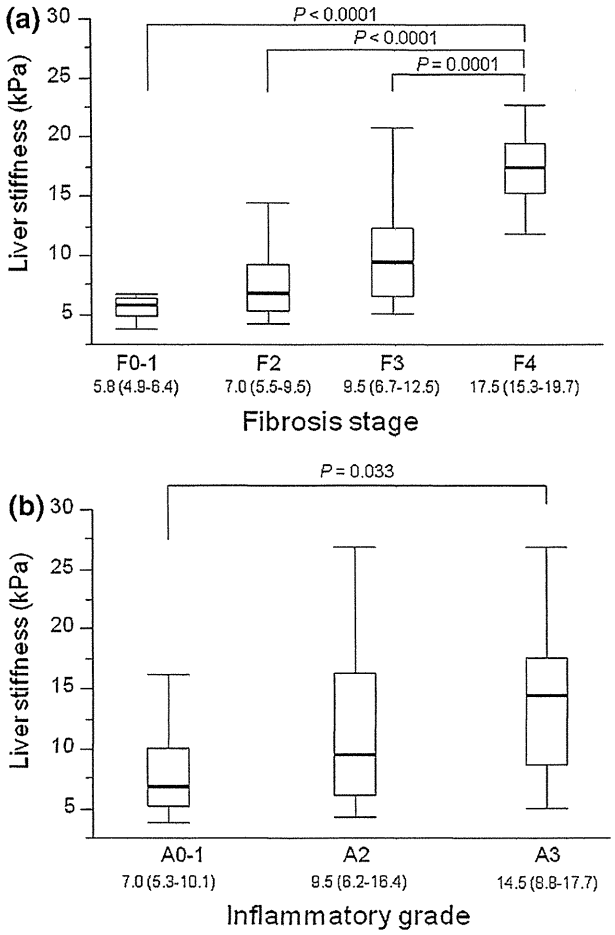

Fig. 2 Liver stiffness (LS) was significantly correlated with fibrosis stage (**a**) and inflammatory grade (**b**). LS significantly differed between F0–1 and F4 ($P < 0.0001$); between F2 and F4 ($P < 0.0001$); and between F3 and F4 ($P = 0.0001$). LS was significantly correlated with fibrosis stage ($\rho = 0.703$, $P < 0.0001$). Inflammatory grade significantly differed between A0–1 and A3 ($P = 0.042$). LS was significantly correlated with inflammatory grade ($\rho = 0.359$, $P = 0.0121$). *Bold lines* are medians; *tops and bottoms of the boxes* are the 25th and 75th percentiles; *horizontal lines* at the ends of the *vertical lines* are the minimum and maximum values observed. *Figures* on the horizontal axis are medians (interquartile range)

Table 3 Cut-off values of liver stiffness for each fibrosis stage (ROC analysis)

	F ≥ 2	F ≥ 3	F = 4
Cut-off value (kPa)	7.1	10.7	16.0
Positive predictive value (%)	100	86.4	91.7
Negative predictive value (%)	33.3	72.4	92.3
Sensitivity (%)	73.3	70.4	78.6
Specificity (%)	100	87.5	97.3
Diagnostic accuracy (%)	76.5	78.4	92.2
Area under ROC curve value	0.844	0.839	0.930

ROC receiver operating characteristics

2 years after the beginning of treatment than at 1 year after the beginning of treatment ($P = 0.0410$). HBV DNA levels were significantly lower at 1 year and at 2 years after the beginning of treatment than at pretreatment ($P < 0.0001$ and $P = 0.0039$).

Changes in fibrosis stages deduced from LS according to cut-off values for fibrosis stages

Fibrosis stages were deduced from LS according to the cut-off LS values for each fibrosis stage. The deduced fibrosis stages of the 1st (pretreatment) and last measurements were compared (Fig. 4).

Seventeen of 21 (81%) patients with deduced fibrosis stages of F2-4 at the 1st measurement had a reduction of deduced stage at the last measurement by antiviral therapy, while none had an increase of the deduced stage. Eleven of 19 (58%) patients with deduced fibrosis stages of F3-4 at the 1st measurement had a 2-point or greater reduction of deduced stage at the last measurement.

The factors associated with a 2-point or greater reduction of deduced fibrosis stage were examined in 19 patients with pretreatment deduced fibrosis stage of F3-4. The change ratio of hyaluronic acid significantly differed between the patients with a 2-point or greater reduction of deduced fibrosis stage and those without such a reduction ($P = 0.0390$) (Table 5).

In patients without antiviral therapy, 11 of 50 (22%) patients with deduced fibrosis stages of F0-3 at the 1st measurement had an increase of the deduced stage, while 8 of 20 (40%) patients with deduced fibrosis stages of F2-4 at the 1st measurement had a reduction of the deduced stage. The factors associated with a 1-point or greater increase in the deduced fibrosis stage were examined in 50 patients with a deduced fibrosis stage of F0-3 at the 1st measurement. Lower baseline albumin levels were associated with a 1-point or greater increase of deduced fibrosis stage ($P = 0.0092$) (Table 6).

Discussion

In the present study, LS was shown to be correlated with fibrosis stage in patients with HBV infection, as has been previously reported. Optimal LS cut-off values with reasonably high sensitivity and specificity were determined to be 7.1 kPa for F ≥ 2, 10.7 kPa for F ≥ 3, and 16.0 kPa for F4, although the negative predictive value for F ≥ 2 was low. In patients with HBV infection, optimal cut-off values of LS have been reported to be 7.2–8.1 kPa for F 2 ≥ and 10.3–13.4 kPa for F4 [21–24]. The cut-off values

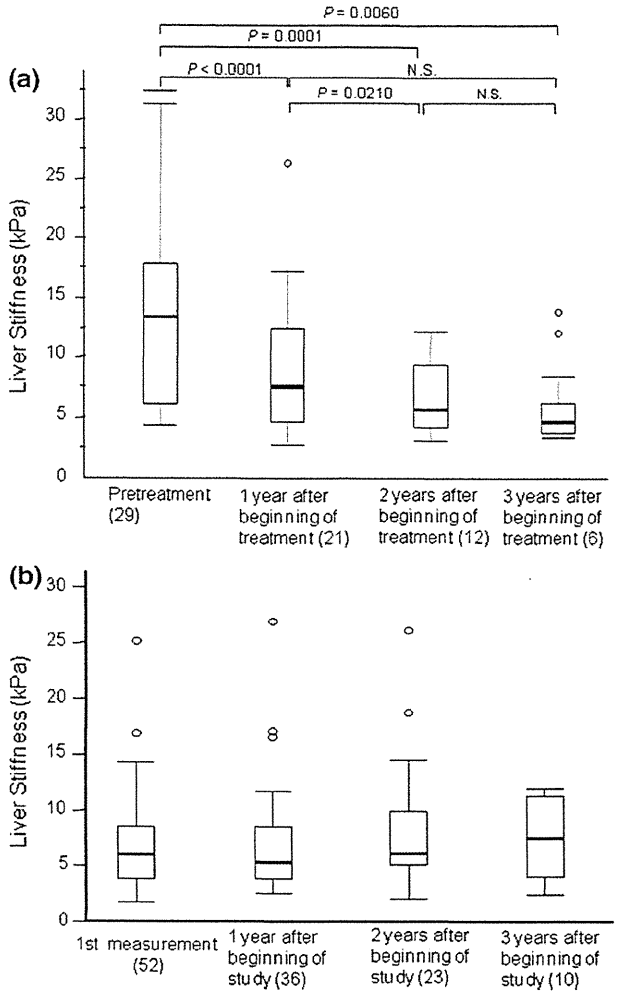

Fig. 3 Changes of liver stiffness (LS) in 29 patients with antiviral therapy (**a**) and 52 patients without antiviral therapy (**b**). **a** In the patients with antiviral therapy, LS values at pretreatment and at 1, 2, and 3 years after the beginning of treatment were 12.9 (6.2–17.9) kPa, 7.5 (5.4–11.7) kPa, 6.5 (5.1–10.6) kPa, and 4.7 (3.1–7.9) kPa, respectively. LS was significantly decreased at 1 year ($P < 0.0001$), 2 years ($P = 0.0001$), and 3 years after the beginning of treatment ($P = 0.0060$) compared with pretreatment values. In addition, LS was significantly decreased at 2 years ($P = 0.0210$) compared with 1 year after the beginning of treatment. **b** In patients without antiviral therapy, LS values of at the 1st measurement and at 1, 2, and 3 years after the beginning of the study were 6.1 (3.9–8.5) kPa, 5.4 (3.7–8.7) kPa, 6.3 (5.2–9.8) kPa, and 7.6 (4.1–11.4) kPa, respectively. No significant difference was observed between any of these values. *Bold lines* are medians; *tops and bottoms of the boxes* are the 25th and 75th percentiles; and *horizontal lines* at the ends of the *vertical lines* are the minimum and maximum values observed

determined in the present study were slightly lower for $F \geq 2$ and slightly higher for F4 compared with those reported previously. Because the number of patients examined in the present study was small, larger studies are needed to confirm the optimal cut-off values of LS for correlating with fibrosis stage in patients with chronic hepatitis B.

The present study showed that LS was correlated with several biological and virological parameters in patients with HBV infection; these parameters included gender, AST, ALT, γ-globulin, total bilirubin, albumin, platelet count, prothrombin time, hyaluronic acid, APRI, FIB-4, HBeAg positivity, and HBV DNA. Mutivariate analysis demonstrated that AST levels, prothrombin time, and hyaluronic acid were independently correlated with LS. Prothrombin time is associated with liver fibrosis and hyaluronic acid is a marker of liver fibrosis. Thus, it is suggested that LS is associated with liver fibrosis. Oliveri et al. [21] reported that, in a multivariate analysis of 171 chronic HBV carriers, fibrosis stage, activity of HBV infection, ALT, and HBV DNA were independently associated with LS. In the present study, the association of LS with AST levels was noted, and this finding indicates the association of LS with inflammatory activity. However, multivariate analysis did not show an association of LS with ALT levels or HBV DNA. The difference between our results and the findings of Oliveri et al. [21] may be attributed to a difference in the population studied, because patients with chronic HBV infection show a variety of pathological states, such as healthy carriers with a high viral load, people with chronic hepatitis, and healthy carriers with a low viral load. Further studies are needed to elucidate the association of LS with ALT and HBV DNA.

The present study showed that antiviral treatment reduced LS. LS was reduced at 1, 2, and 3 years after the beginning of antiviral treatment compared with the pretreatment values. In addition, it was reduced at 2 years after the beginning of antiviral treatment compared with the values at 1 year after the beginning of treatment. Enomoto et al. [25] reported that LS was significantly decreased in patients with chronic hepatitis B by 1 year of therapy with entecavir. The present results indicate that LS continues to reduce from 1 year after the beginning of treatment.

Attenuation of liver fibrosis has been reported in 35–38% of patients with chronic hepatitis B with lamivudine and in 36–39% with entecavir after 1 year of treatment [29, 30]. Attenuation of necroinflammatory activity was also noted in 61–62% of patients with chronic hepatitis B with lamivudine and in 70–72% with entecavir [31, 32]. Long-term treatment (more than 3 years) with entecavir was reported to attenuate necroinflammatory activity in all the patients and to attenuate liver fibrosis in 57–100% of the patients [31, 32]. In the present study, 17 of 21 (81%) patients with deduced fibrosis stages of F2-4 had a reduction of the deduced stage by antiviral therapy after 1.5 years, while none had an increase of the deduced stage. Eleven of 19 (58%) patients with deduced fibrosis stages of F3-4 had a 2-point or greater reduction of the deduced stage. The proportion of patients with a reduction of

Table 4 Changes in biochemical and serological parameters during antiviral therapy

	Pretreatment	1 year after beginning of treatment	2 years after beginning of treatment	3 years after beginning of treatment
AST (IU/L)	50.0 (38.5–77.0)[a]	24.5 (19.8–36.3)[a]	21.0 (18.0–26.0)[a]	20.0 (18.0–25.0)[a]
ALT (IU/L)	62.0 (33.0–109.0)[b]	23.0 (14.0–30.8)[b]	18.0 (13.0–20.0)[b]	18.0 (14.0–26.0)[b]
Platelet count ($\times 10^4/\mu L$)	12.2 (9.1–17.0)	12.3 (10.1–17.8)[c]	13.5 (10.4–17.0)[c]	12.6 (10.0–20.9)
HBV DNA (log copy/mL)	6.7 (4.1–7.2)[d]	2.6[d]	2.6[d]	2.6 (2.6–2.9)

Values are medians (interquartile ranges)

AST aspartate aminotransferase, *ALT* alanine aminotransferase

[a] AST levels were significantly lower at 1 year, at 2 years, and at 3 years after the beginning of treatment than at pretreatment ($P = 0.0002$, $P = 0.0039$, and $P = 0.0313$). AST level was significantly lower at 2 years after the beginning of treatment than at 1 year after the beginning of treatment, ($P = 0.0024$)

[b] ALT levels were significantly lower at 1 year, at 2 years, and at 3 years after the beginning of treatment than at pretreatment ($P = 0.0001$, $P = 0.0078$ and $P = 0.0313$). ALT level was significantly lower at 2 years after the beginning of treatment than at 1 year after the beginning of treatment, ($P = 0.0081$)

[c] Platelet count was significantly higher at 2 years after the beginning of treatment than at 1 year after the beginning of treatment ($P = 0.0410$)

[d] HBV DNA levels were significantly lower at 1 year and at 2 years after the beginning of treatment than at pretreatment ($P < 0.0001$ and $P = 0.0039$)

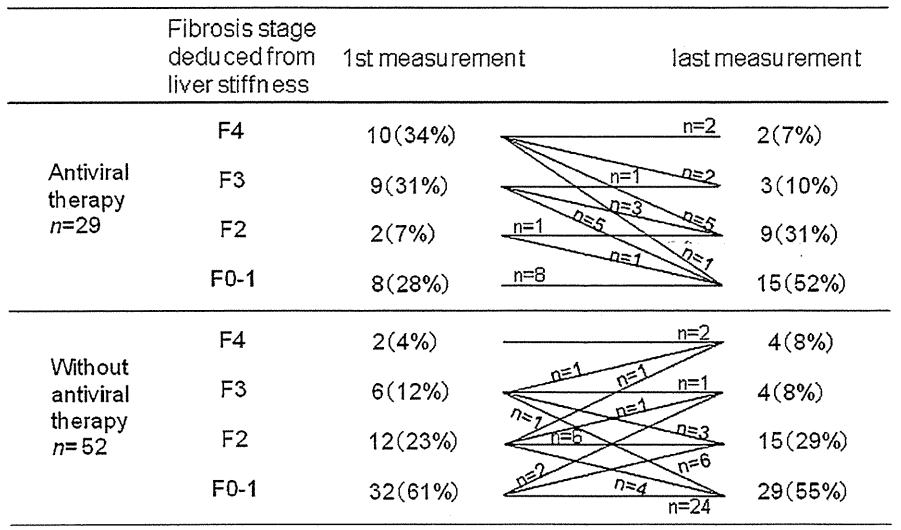

Fig. 4 Changes of fibrosis stages deduced from liver stiffness (LS) according to cut-off values for fibrosis stages. Seventeen of 21 (81%) patients with deduced fibrosis stages of F2-4 at the 1st measurement had a reduction of deduced stage by antiviral therapy, while none of these patients had an increase of the deduced stage at the last LS measurement. Eleven of 19 (58%) patients with deduced fibrosis stages of F3-4 at the 1st measurement had a 2-point or greater reduction of deduced stage at the last LS measurement. The interval between the pretreatment measurement and the last measurement of LS was 512 (range 366–728) days. In patients without antiviral therapy, 11 of 50 (22%) patients with deduced fibrosis stages of F0-3 at the 1st LS measurement had an increase of the deduced stage, while 8 of 20 (40%) patients with deduced fibrosis stages of F2-4 at the 1st measurement had a reduction of the deduced stage. The interval between the 1st LS measurement and the last measurement was 422 (range 358–709) days

deduced fibrosis stage in the present study is similar to that in previous reports based on biopsy. Thus, it seems that LS measurement is useful to monitor the regression of fibrosis by antiviral treatment.

We found that a 2-point or greater reduction of deduced fibrosis stage was significantly associated with a reduction of hyaluronic acid. In the present study, no liver biopsy was done after antiviral therapy. A reduction of deduced fibrosis stage can be attributed not only to reduction of fibrosis but also to a reduction of necroinflammatory activity. However, the finding that the reduction of deduced fibrosis stage was correlated with a reduction of hyaluronic acid but not with a reduction of ALT levels indicates that reduction of the deduced fibrosis stage can be attributed to